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Short Communication

The Importance of Oxidative Stress and the Microbiome in Initiation of Chronicity

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INTRODUCTION

Chronic wounds develop as a result of defective regulation of the complex cellular and molecular processes involved in proper healing [1–4]. Chronic wounds affect nearly 8.2 million people worldwide and cost between $28 to $31 billion annually to treat [5]. Although a number of studies have been performed to address the processes involved in wound chronicity (including genetic, non-genetic, and epigenetic processes), so far, the scientific wound healing community has been unable to crack the very difficult, complex, and multi-dimensional processes involved in initiation/development of chronic wounds and, in particular, of diabetic ulcers. This is primarily because we cannot experiment in humans and it is virtually impossible to study the initiation and development of wound chronicity in humans because by the time such wounds present themselves in the clinic, initial stages of development are long gone. Therefore, animal models for study of the genesis of non-healing chronic wounds and of progression to full chronicity are critical for elucidating the processes involved.

Although several animal models have been previously developed to study chronic wounds, these models only mimic some of the critical elements of chronic wounds in humans [6-10]. This includes venous ulcers, pressure ulcers, wounds resulting from ischemia-reperfusion conditions, and those in which investigators introduce biofilm-forming bacteria to cause wounds to become chronic [11-20]. More recently, wounds in a diabetic mouse model induced by streptozotocin were challenged with *Pseudomonas aeroginosa* to induce chronicity [21]. This model mimics some aspects of type I diabetes in humans but the wounds do not attain the characteristics of chronicity found in wounds of patients with type II diabetes, which are the most severe and common type of chronic wounds. Indeed, these models involved infecting the wounds with biofilm-forming bacteria maintained in the laboratory. This situation is not representative of human chronic wounds in which biofilm occurs spontaneously and is derived from the skin microbiome. Therefore, more appropriate models need to be developed.

A MOUSE MODEL TO STUDY CHRONIC WOUND DEVELOPMENT AND PROGRESSION

We have recently developed a chronic wound model in the db/db$^{-/-}$ type II diabetic mouse [6]. To develop this model we took advantage of the fact that chronic wounds in humans contain toxic concentrations of Reactive Oxygen Species (ROS) and biofilm-producing bacteria [22–25]. It has been shown that ROS impair keratinocyte migration *in vitro*, potentially inhibiting re-epithelialization [26,27] and that high levels of ROS also lead to DNA damage, gene dysregulation, protein and lipid damage, a hostile proteolytic environment, and cell death [6,25].

To generate chronic wounds in the db/db$^{-/-}$ mice, we create high levels of Oxidative Stress (OS) in the wound tissue with a single treatment with specific inhibitors for the antioxidant enzymes catalase and GPx at the time of wounding (Figure 1). The wounds become chronic within 20 days after treatment and remain chronic for 40-100 days if the animal survives [6,28,29]. In addition to lack of closure, excessive inflammation, lack of blood flow, high proteolytic environment, and poor matrix deposition, these wounds spontaneously develop a complex microbiota of the same biofilm-forming bacteria that are found in chronic wounds in humans, leading to the chronic presence of biofilm (Figure 1). Furthermore, when we treated the chronic wounds starting at 20 days post-wounding with N-Acetyl-L-Cysteine (NAC) and α-tocopherol, two antioxidant agents, we can reverse chronicity [6]. We found that the OS falls rapidly, the biofilm dismantles, and the wounds heal, strongly suggesting that the high levels of OS we created immediately after wounding is important for initiation of chronicity [6]. In addition, we have also found experimentally using our chronic wound model that high OS is necessary but not sufficient for chronic wound development. The microbiome of the skin is critical for the development of biofilm in the presence of high OS [30,31].

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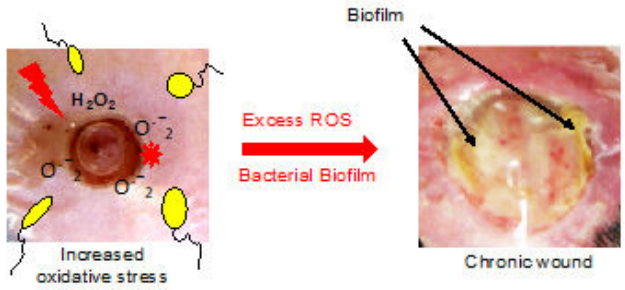

Figure 1: Oxidative Stress (OS) in the wound tissue with a single treatment with specific inhibitors for the antioxidant enzymes catalase and GPx at the time of wounding.

POTENTIAL FOR NOVEL TREATMENT APPROACHES

Numerous attempts in treating chronic wounds have consistently been made in the last couple of decades, but success has been limited. Using our model in diabetic mice, we have analyzed various processes involved in initiation of chronicity over the first 48 hour post-injury. Our data indicate that during the first 48 hour post-injury, levels of oxidative stress and inflammation in chronic wounds are critical for initiation of chronic wound development [32]. In addition, elevated levels of epinephrine in the wound prevent re-epithelialization and alterations found during the hypoxic processes strongly suggest that the triggers for angiogenesis do not occur; indeed, they are suppressed, and therefore the wound is deprived of nutrients and oxygen. As a consequence, the building blocks needed for ATP production, the energy currency of the cell, are not present in sufficient levels to allow for proper cell function needed for granulation tissue formation and re-epithelialization. Under these conditions, cell death ensues and the wound healing processes are halted [32].

CONCLUSION

Given our findings in these studies, we propose that during the first 24hr after debridement, the wound be treated with:

- Both antioxidant molecules that eliminate reactive oxidative species and with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) to decrease oxidative stress caused by pro-inflammatory lipid molecules;
- An activator(s) of Nrf2;
- An inhibitor(s) of Hif3α; and
- An inhibitor(s) of epinephrine to allow re-epithelialization to initiate.

At 24hr, the wound should be treated in addition with:

- PDGF, VEGF, and an inhibitor(s) of Thbs1 to stimulate angiogenesis;
- Inhibitors of cathepsins and ECM degrading enzymes to decrease matrix degradation; and
- Addition of IL-6 to stimulate the activation of M2 macrophages.

Furthermore, the patient should be treated immediately with products that stimulate energy production to increase the levels of ATP in cells, and with anti-lipid peroxidation molecules to decrease cell membrane damage. In addition, because the microbiome of the skin is critical for biofilm formation, the wounds should also be examined for the composition of the microbiome so that appropriate treatments are applied to prevent the return of biofilm. Decreasing the oxidative stress with antioxidants should help decrease the probabilities of biofilm returning but in addition, treatment with small molecules that penetrate bacterial cells, halts protein synthesis, and leads to bacteria cell death should also be considered in conjunctions with topical antibiotics. Such treatment approach should continue until it is clear that the wound tissue is forming and re-epithelialization is occurring. The time needed for this combined treatment will have to be evaluated by the physician to personalize treatment.

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